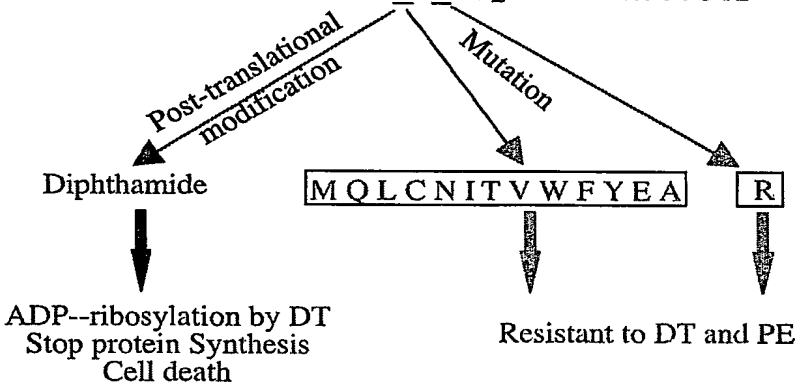
H. sapiens(66%)	...706 DVTLHADAIHRGGGQIIPTARR...858	SEQ ID NO:1
M. musculus(3'mRNA)	..... DVTLHADAIHRGGGQIIPTARR...	SEQ ID NO:2
R. norvegicus(66%)	...706 DVTLHADAIHRGGGQIIPTARR...858	SEQ ID NO:3
C. griseus(CHO, 67%)	...706 DVTLHADAIHRGGGQIIPTARR...858	SEQ ID NO:4
D. melanogaster(67%)	...692 DVTLHADAIHRGGGQIIPTTRR...848	SEQ ID NO:5
C. elegans (67%)	...700 DVTLHADAIHRGGGQIIPTARR...852	SEQ ID NO:6
S. pombe (79%)	...690 DVVLHADAIHRGGGQIIPTARR...842	SEQ ID NO:7
P. pastoris (88%)	...690 DVTLHADAIHRGGGQVIPTMKR...842	SEQ ID NO:8
S. cerevisiae	...690 DVTLHADAIHRGGGQIIPTMRR...842	SEQ ID NO:9




```
2070        2079        2088        2097        2106        2115        2124        2133
                                                 R
                                            cGc cGc GGA
 GAT GTT ACC CTG CAC GCC GAT GCT ATC CAC AGA GGT GGA GGA CAA GTC ATT CCA ACC ATG AAG AGA
   D   V   T   L   H   A   D   A   I   H   R   G   G   G   Q   V   I   P   T   M   K   R
690                                      700                                     710
```

SEQ ID NO:10

FIG. 1

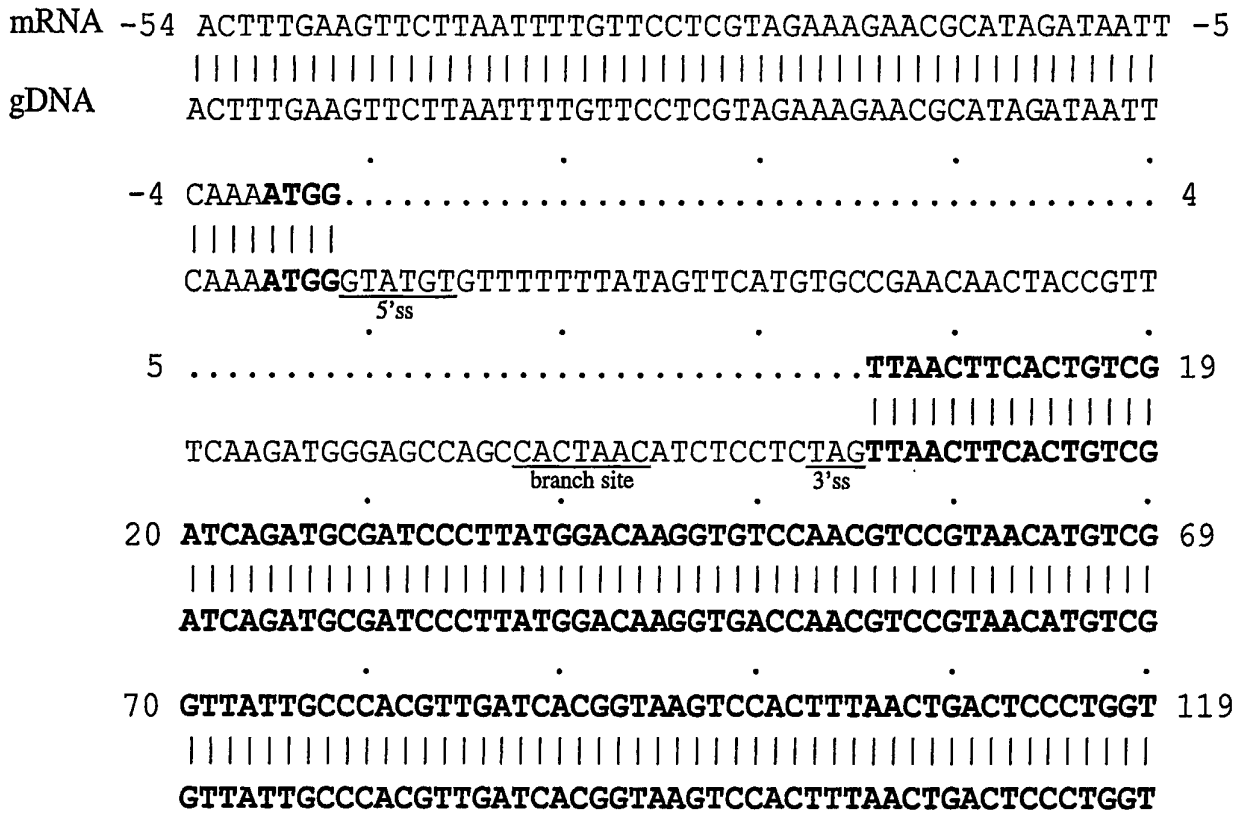

mRNA -54 ACTTTGAAGTTCTTAATTTTGTTCCTCGTAGAAAGAACGCATAGATAATT -5
gDNA ACTTTGAAGTTCTTAATTTTGTTCCTCGTAGAAAGAACGCATAGATAATT
-4 CAAAATGG 4
CAAAATGGGTATGTGTTTTTTTATAGTTCATGTGCCGAACAACTACCGTT
5'ss
5 TTAACTTCACTGTCG 19
TCAAGATGGGAGCCAGCCACTAACATCTCCTCTAGTTAACTTCACTGTCG
branch site
3'ss
20 ATCAGATGCGATCCCTTATGGACAAGGTGTCCAACGTCCGTAACATGTCG 69
ATCAGATGCGATCCCTTATGGACAAGGTGACCAACGTCCGTAACATGTCG
70 GTTATTGCCCACGTTGATCACGGTAAGTCCACTTTAACTGACTCCCTGGT 119
GTTATTGCCCACGTTGATCACGGTAAGTCCACTTTAACTGACTCCCTGGT

FIG. 2

```
          -51        -41        -31        -21        -11        -1
          GATACTT TGAAGTTCTT AATTTTGTTC CTCGTAGAAA GAACGCATAG ATAATTCAAA

          9          18         27         36         45         54
ATG GTT AAC TTC ACT GTC GAT CAG ATG CGA TCC CTT ATG GAC AAG GTG ACC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   V   N   F   T   V   D   Q   M   R   S   L   M   D   K   V   T   N

          63         72         81         90         99         108
GTC CGT AAC ATG TCG GTT ATT GCC CAC GTT GAT CAC GGT AAG TCC ACT TTA ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   R   N   M   S   V   I   A   H   V   D   H   G   K   S   T   L   T

          117        126        135        144        153        162
GAC TCC CTG GTG CAA CGT GCC GGT ATT ATT TCT GCT GCC AAG GCT GGT GAG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   L   V   Q   R   A   G   I   I   S   A   A   K   A   G   E   A

          171        180        189        198        207        216
CGT TTC ACT GAT ACT AGA AAG GAC GAG CAA GAG AGA GGT ATC ACC ATC AAG TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   F   Ⓣ   D   T   R   K   D   E   Q   E   R   G   I   T   I   K   S

          225        234        243        252        261        270
ACC GCC ATT TCT TTG TAC TCT GAG ATG GGT GAC GAC GAT GTC AAG GAG ATC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   A   I   S   L   Y   S   E   M   G   D   D   D   V   K   E   I   K

          279        288        297        306        315        324
CAG AAG ACT GAA GGT AAC AGT TTC CTT ATC AAC TTA ATT GAC TCC CCA GGT CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   K   T   E   G   N   S   F   L   I   N   L   I   D   S   P   G   H

          333        342        351        360        369        378
GTT GAC TTC TCT TCT GAG GTC ACT GCT GCT CTG CGT GTT ACT GAC GGT GCT TTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   D   F   S   S   E   V   T   A   A   L   R   V   T   D   G   A   L

          387        396        405        414        423        432
GTC GTC GTT GAC TGT GTT GAA GGT GTC TGT GTT CAA ACT GAG ACC GTT TTG CGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   V   V   D   C   V   E   G   V   C   V   Q   T   E   T   V   L   R

          441        450        459        468        477        486
CAA GCT TTG GGT GAA AGA ATC AAG CCA GTT GTT GTC ATT AAC AAG GTC GAC CGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   A   L   G   E   R   I   K   P   V   V   V   I   N   K   V   D   R

          495        504        513        522        531        540
GCT CTT TTG GAG TTG CAA GTT ACC AAG GAG GAC CTG TAC CAG TCT TTC GCT AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   L   L   E   L   Q   V   T   K   E   D   L   Y   Q   S   F   A   R

          549        558        567        576        585        594
ACC GTC GAG TCC GTA AAC GTC GTT ATC GCT ACT TAC ACT GAC AAG ACC ATT GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   V   E   S   V   N   V   V   I   A   T   Y   T   D   K   T   I   G

          603        612        621        630        639        648
GAC AAC CAA GTC TAC CCA GAA CAG GGT ACC GTC GCT TTC GGT TCA GGT CTG CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   N   Q   V   Y   P   E   Q   G   T   V   A   F   G   S   G   L   H

          657        666        675        684        693        702
GGA TGG GCT TTC ACC GTT AGA CAG TTC GCC ACT AGA TAC TCC AAG AAG TTC GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   W   A   F   T   V   R   Q   F   A   T   R   Y   S   K   K   F   G
```

FIG. 3A

```
        711         720         729         738         747         756
GTT GAC AGA ATC AAG ATG ATG GAG CGT CTG TGG GGA GAC TCT TAC TTC AAC CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   D   R   I   K   M   M   E   R   L   W   G   D   S   Y   F   N   P

        765         774         783         792         801         810
AAG ACC AAG AAA TGG ACC AAC AAG GAC AAG GAC GCC GCT GGA AAG CCT TTG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   T   K   K   W   T   N   K   D   K   D   A   A   G   K   P   L   E

        819         828         837         846         855         864
CGT GCC TTC AAC ATG TTC GTT TTG GAC CCT ATC TTC CGT CTG TTT GCT GCC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   A   F   N   M   F   V   L   D   P   I   F   R   L   F   A   A   I

        873         882         891         900         909         918
ATG AAC TTC AAG AAG GAT GAA ATT CCA GTT CTG TTG GAG AAA TTG GAG ATC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   N   F   K   K   D   E   I   P   V   L   L   E   K   L   E   I   N

        927         936         945         954         963         972
CTG AAG CGT GAG GAG AAG GAG TTG GAG GGT AAG GCT CTT TTG AAG GTT GTC ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   K   R   E   E   K   E   L   E   G   K   A   L   L   K   V   V   M

        981         990         999        1008        1017        1026
AGA AAG TTC TTG CCA GCT GCC GAC GCT TTG TTG GAG ATG ATT GTT CTT CAC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   K   F   L   P   A   A   D   A   L   L   E   M   I   V   L   H   L

       1035        1044        1053        1062        1071        1080
CCA TCT CCA GTC ACC GCT CAA GCT TAC AGA GCC GAG ACT TTG TAC GAA GGT CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   P   V   T   A   Q   A   Y   R   A   E   T   L   Y   E   G   P

       1089        1098        1107        1116        1125        1134
TCT GAT GAC CAA TTC TGC ATT GGT ATC AGA GAG TGT GAC CCT AAG GCT GAG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   D   D   Q   F   C   I   G   I   R   E   C   D   P   K   A   E   L

       1143        1152        1161        1170        1179        1188
ATG GTT TAC ATT TCC AAG ATG GTG CCA ACC TCC GAC AAA GGT AGA TTC TAC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   V   Y   I   S   K   M   V   P   T   S   D   K   G   R   F   Y   A

       1197        1206        1215        1224        1233        1242
TTC GGT CGT GTT TTC TCC GGT ACT GTT AAG TCC GGT CAA AAG GTC AGA ATC CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   G   R   V   F   S   G   T   V   K   S   G   Q   K   V   R   I   Q

       1251        1260        1269        1278        1287        1296
GGT CCT AAC TAC GTT CCA GGT AAG AAG GAG GAC TTG TTC ATC AAG GCT GTT CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   P   N   Y   V   P   G   K   K   E   D   L   F   I   K   A   V   Q

       1305        1314        1323        1332        1341        1350
AGA ACT GTT TTG ATG ATG GGA AGA ACC GTC GAG CCT ATT GAC GAT GTC CCA GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   T   V   L   M   M   G   R   T   V   E   P   I   D   D   V   P   A

       1359        1368        1377        1386        1395        1404
GGT AAC ATT CTG GGT ATT GTG GGT ATC GAC CAG TTC TTG CTG AAG TCT GGT ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   N   I   L   G   I   V   G   I   D   Q   F   L   L   K   S   G   T

                    Δ5'EF-2
       1413        1422        1431        1440        1449        1458
CTT ACT ACC AAC GAA GCC GCT CAC AAC ATG AAG GTG ATG AAA TTC TCT GTC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   T   N   E   A   A   H   N   M   K   V   M   K   F   S   V   S
```

FIG. 3B

```
      1467        1476        1485        1494        1503        1512
CCA GTT GTG CAA GTT GCC GTT GAG GTC AAG AAC GCT AAT GAT CTG CCC AAG TTG
---------------------------------------------------------------------
 P   V   V   Q   V   A   V   E   V   K   N   A   N   D   L   P   K   L

      1521        1530        1539        1548        1557        1566
GTT GAG GGT CTG AAG CGT TTG TCC AAG TCT GAC CCA TGT GTT TTA ACC TAC ATC
---------------------------------------------------------------------
 V   E   G   L   K   R   L   S   K   S   D   P   C   V   L   T   Y   I

      1575        1584        1593        1602        1611        1620
TCC GAG TCT GGT GAG CAC ATT GTT GCT GGT ACT GGT GAG CTG CAC TTG GAA ATC
---------------------------------------------------------------------
 S   E   S   G   E   H   I   V   A   G   T   G   E   L   H   L   E   I

      1629        1638        1647        1656        1665        1674
TGT TTG CAA GAT CTG CAA GAC GAC CAC GCT GGT GTC CCT CTG AAG ATT TCT CCT
---------------------------------------------------------------------
 C   L   Q   D   L   Q   D   D   H   A   G   V   P   L   K   I   S   P

      1683        1692        1701        1710        1719        1728
CCA GTT GTT ACC TAC CGT GAG ACT GTC ACT AAC GAA TCT TCC ATG ACT GCC CTG
---------------------------------------------------------------------
 P   V   V   T   Y   R   E   T   V   T   N   E   S   S   M   T   A   L

      1737        1746        1755        1764        1773        1782
TCC AAG TCT CAG AAC AAG CAT AAC AGA ATT TAC CTG AAG GCT CAA CCA ATT GAC
---------------------------------------------------------------------
 S   K   S   Q   N   K   H   N   R   I   Y   L   K   A   Q   P   I   D

      1791        1800        1809        1818        1827        1836
GAG GAA TTG TCT TTG GCT ATC GAA GAA GGT AAG GTT CAC CCA AGA GAC GAC TTT
---------------------------------------------------------------------
 E   E   L   S   L   A   I   E   E   G   K   V   H   P   R   D   D   F

      1845        1854        1863        1872        1881        1890
AAA GCC AGA GCC AGA ATC ATG GCT GAT GAA TAC GGT TGG GAC GTC ACT GAT GCC
---------------------------------------------------------------------
 K   A   R   A   R   I   M   A   D   E   Y   G   W   D   V   T   D   A

      1899        1908        1917        1926        1935        1944
AGA AAG ATC TGG TGT TTC GGT CCA GAC GGT ACT GGT GCC AAC TTA GTT GTT GAC
---------------------------------------------------------------------
 R   K   I   W   C   F   G   P   D   G   T   G   A   N   L   V   V   D

      1953        1962        1971        1980        1989        1998
CAG TCT AAG GCT GTC CAA TAC TTG CAC GAG ATC AAG GAC TCT GTT GTT GCC GGT
---------------------------------------------------------------------
 Q   S   K   A   V   Q   Y   L   H   E   I   K   D   S   V   V   A   G

      2007        2016        2025        2034        2043        2052
TTC CAA TTG GCT ACC AAG GAA GGT CCA ATT TTG GGA GAA AAC ATG AGA TCC GTC
---------------------------------------------------------------------
 F   Q   L   A   T   K   E   G   P   I   L   G   E   N   M   R   S   V

      2061        2070        2079        2088        2097        2106
AGA GTC AAC ATC TTG GAT GTT ACC CTG CAC GCC GAT GCT ATC CAC AGA GGT GGA
---------------------------------------------------------------------
 R   V   N   I   L   D   V   T   L   H   A   D   A   I   H   R   G   G

      2115        2124        2133        2142        2151        2160
GGA CAA GTC ATT CCA ACC ATG AAG AGA GTT ACC TAC GCC GCC TTC CTG TTG GCT
---------------------------------------------------------------------
 G   Q   V   I   P   T   M   K   R   V   T   Y   A   A   F   L   L   A

      2169        2178        2187        2196        2205        2214
GAG CCA GCT ATC CAG GAG CCT ATC TTC TTG GTG GAG ATC CAA TGT CCA GAG AAT
---------------------------------------------------------------------
 E   P   A   I   Q   E   P   I   F   L   V   E   I   Q   C   P   E   N
```

FIG. 3C

```
      2223        2232        2241        2250        2259        2268
GCC ATT GGT GGT ATC TAC TCT GTT TTG AAC AAG AAG AGA GGT CAA GTT ATC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   I   G   G   I   Y   S   V   L   N   K   K   R   G   Q   V   I   S

      2277        2286        2295        2304        2313        2322
GAG GAA CAA AGA CCA GGT ACC CCA TTG TTC ACT GTC AAA GCT TAC TTG CCA GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   E   Q   R   P   G   T   P   L   F   T   V   K   A   Y   L   P   V

      2331        2340        2349        2358        2367        2376
AAC GAG TCA TTC GGT TTC ACC GGT GAA CTG AGA CAA GCT ACC GCT GGT CAA GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   E   S   F   G   F   T   G   E   L   R   Q   A   T   A   G   Q   A

      2385        2394        2403        2412        2421        2430
TTC CCA CAG ATG GTG TTC GAC CAC TGG GCC AAC ATG AAT GGT AAC CCA TTG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   P   Q   M   V   F   D   H   W   A   N   M   N   G   N   P   L   D

      2439        2448        2457        2466        2475        2484
CCA GCC TCC AAG GTC GGT GAG ATT GTT CTT GCT GCC AGA AAG AGA CAG GGT ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   A   S   K   V   G   E   I   V   L   A   A   R   K   R   Q   G   M

      2493        2502        2511        2520        2529        2538
AAG GAG AAC GTT CCT GGT TAT GAA GAG TAC TAC GAC AAG TTG TAA GCT TAA TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   E   N   V   P   G   Y   E   E   Y   Y   D   K   L

      2547        2556        2565        2574        2583        2592
TTC ATT AAC TTA TTT GTG TCG TTC GTA TGT CTA TTT ACG TAC TTA ATT CAG TGT
     A5'EF-2

      2601
ATT GTT GTT 3'
```

FIG. 3D

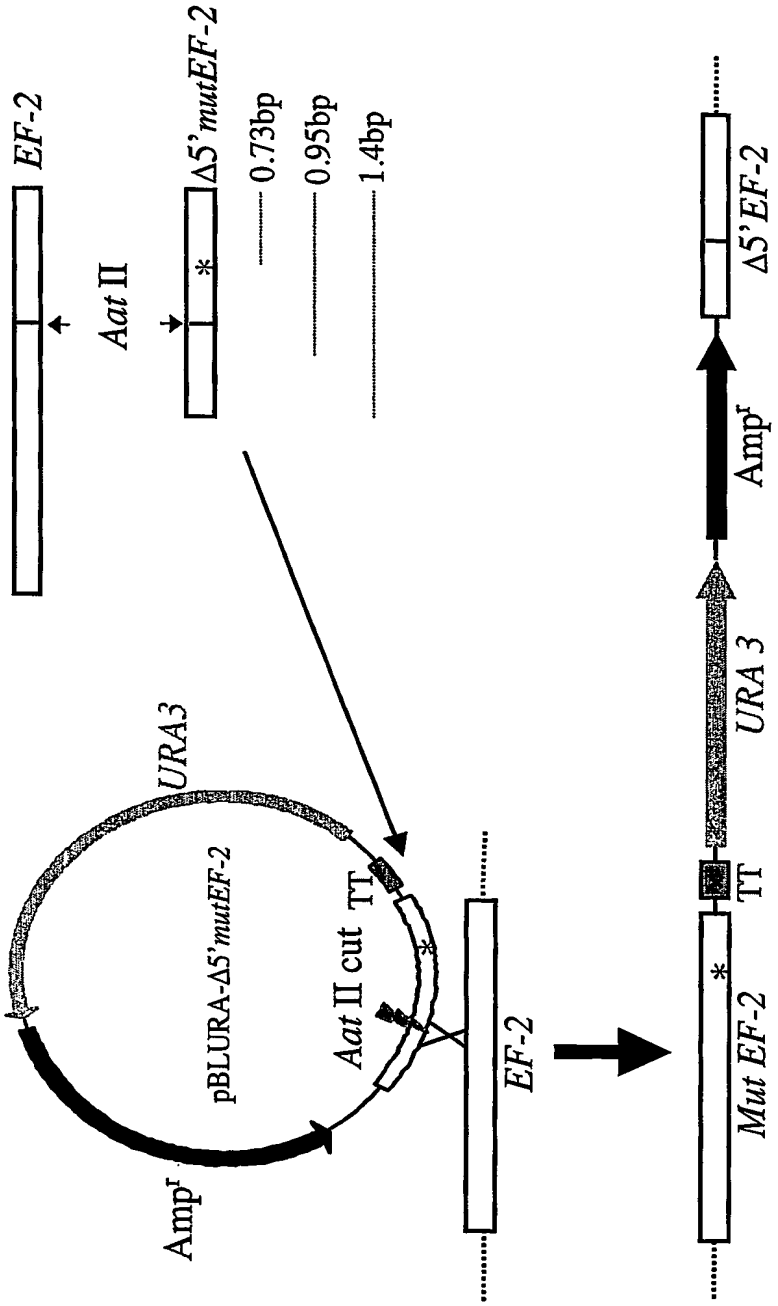

EF-2
Aat II
Δ5'mutEF-2
0.73bp
0.95bp
1.4bp
URA3
pBLURA-Δ5'mutEF-2
Aat II cut
TT
Ampr
EF-2
Mut EF-2
TT
URA 3
Ampr
Δ5'EF-2

FIG. 4

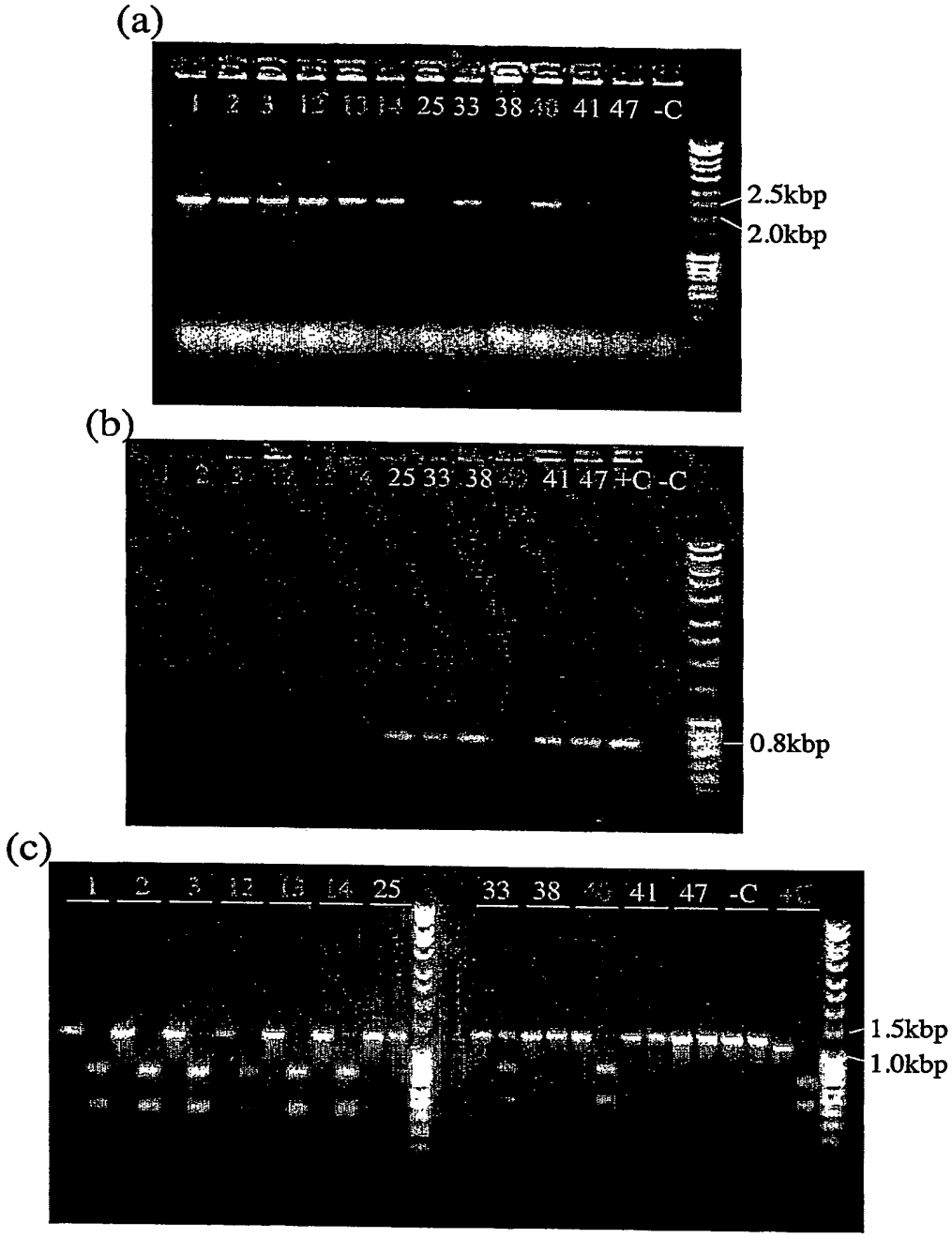


FIG. 5

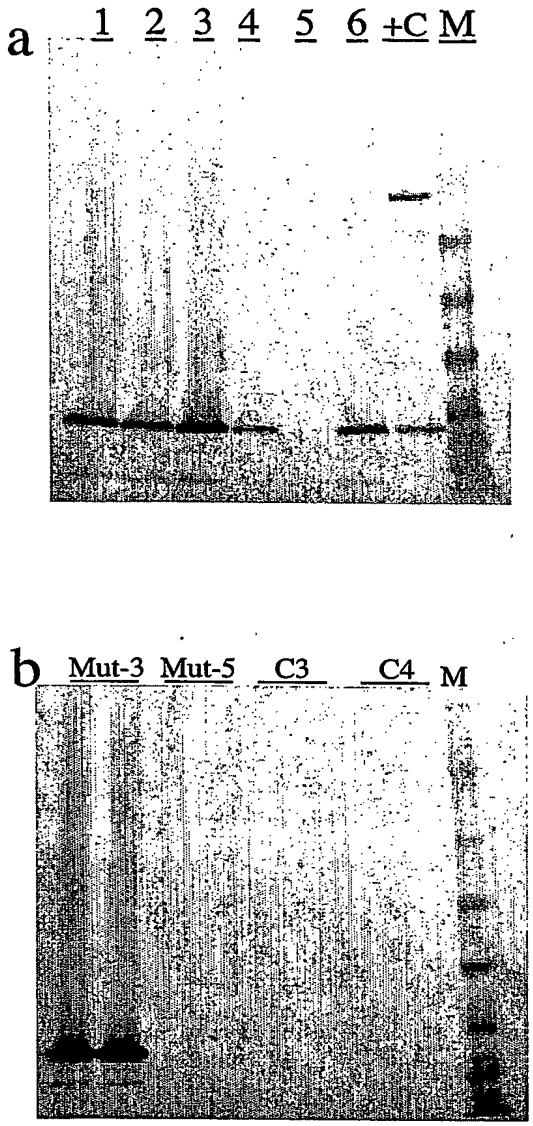


FIG. 6

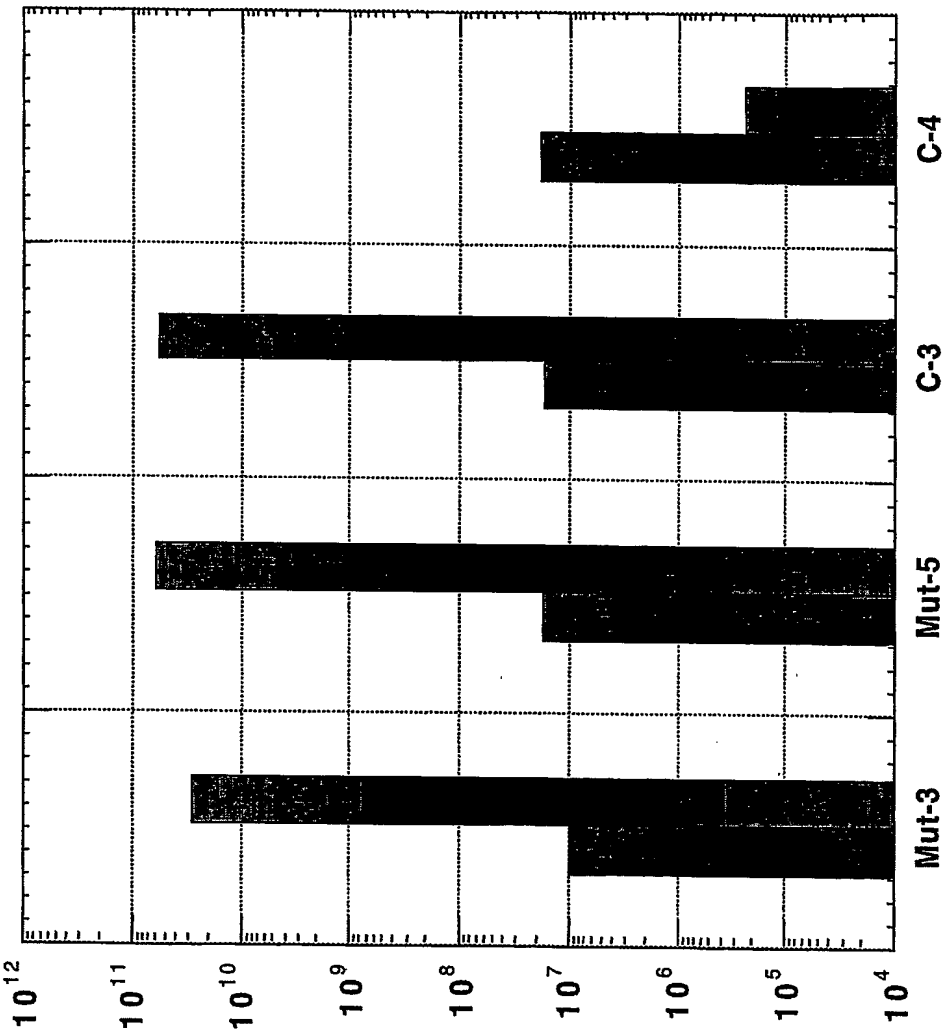


FIG. 7

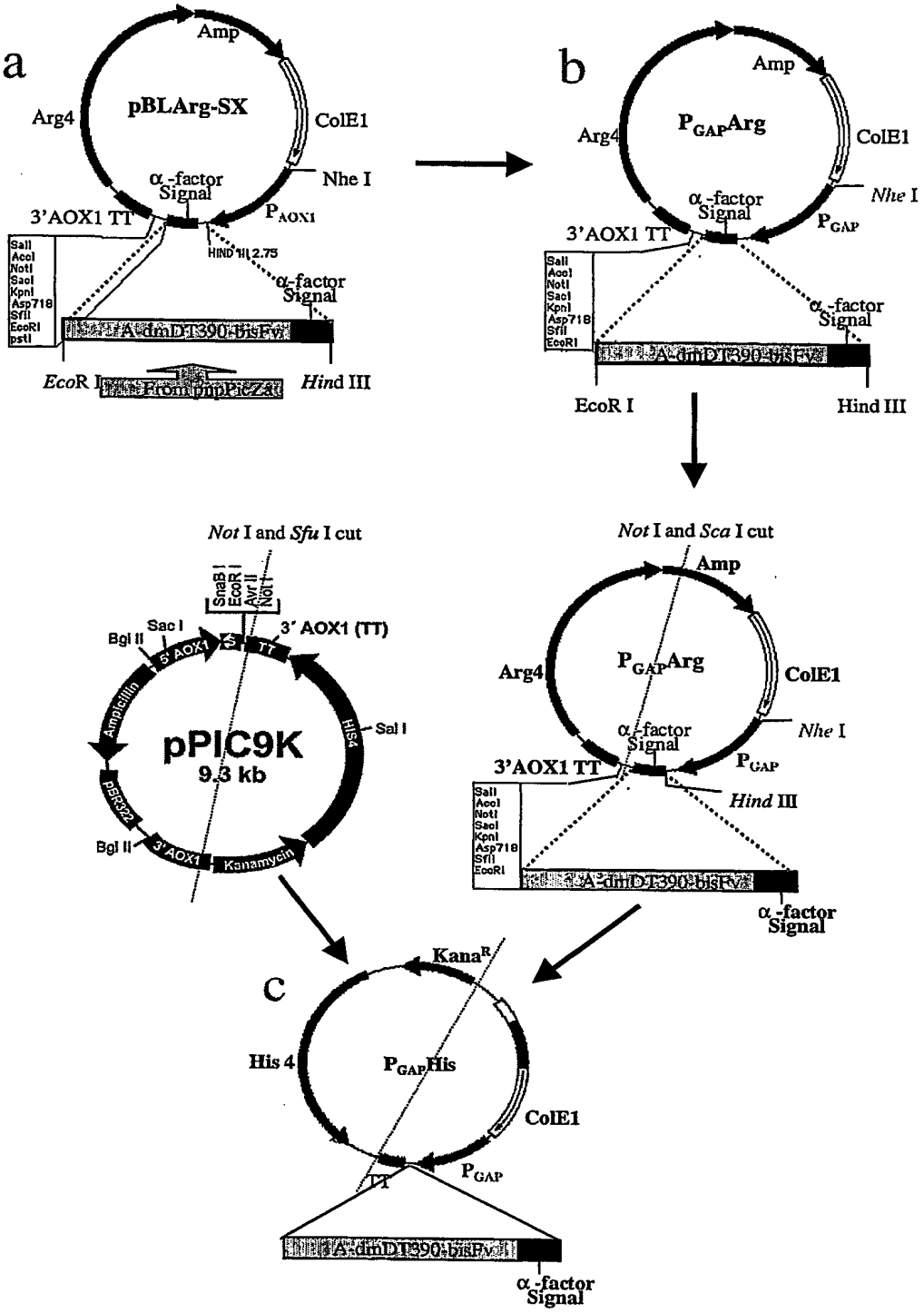

a
Amp
pBLArg-SX
Arg4
ColE1
α-factor Signal
Nhe I
3'AOX1 TT
P AOX1
HIND III 2.75
α-factor Signal
Sall
AccI
NotI
SacI
KpnI
Asp718
SfiI
EcoRI
pstI
A-dmDT390-bisFv
EcoR I
From pppPicZα
Hind III
b
Amp
P GAP Arg
Arg4
ColE1
α-factor Signal
Nhe I
3'AOX1 TT
P GAP
α-factor Signal
EcoR I
Hind III
Not I and Sfu I cut
SnaB I
EcoR I
Avr II
Not I
Bgl II
Sac I
3' AOX1 (TT)
5' AOX1
TT
Ampicillin
pPIC9K
9.3 kb
His4
Sal I
pBR322
3'AOX1
Kanamycin
Bgl II
Not I and Sca I cut
Amp
P GAP Arg
Arg4
ColE1
α-factor Signal
Nhe I
3'AOX1 TT
P GAP
Hind III
α-factor Signal
C
Kana R
His 4
P GAP His
ColE1
TT
P GAP
α-factor Signal

FIG. 8

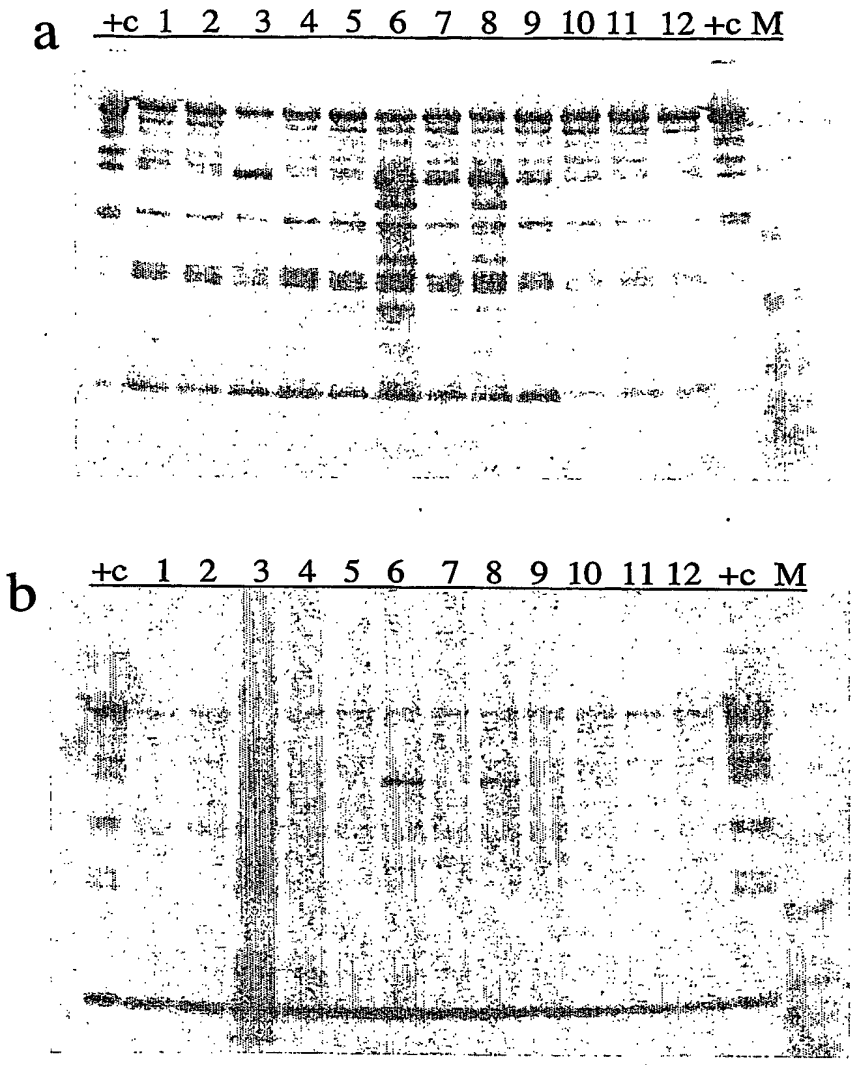


FIG. 9

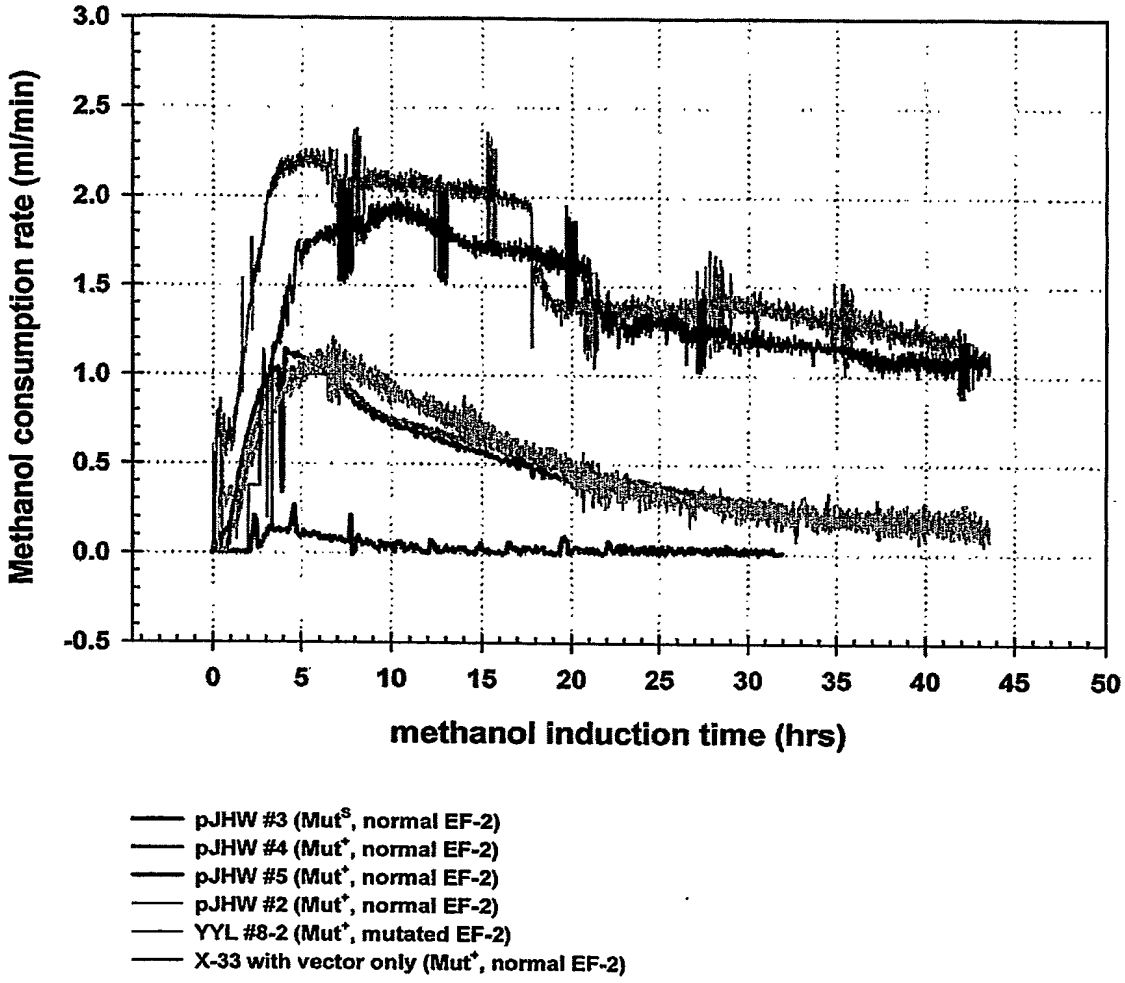

Methanol consumption rate (ml/min)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
5
10
15
20
25
30
35
40
45
50
methanol induction time (hrs)
pJHW #3 (Mut^S, normal EF-2)
pJHW #4 (Mut$^+$, normal EF-2)
pJHW #5 (Mut$^+$, normal EF-2)
pJHW #2 (Mut$^+$, normal EF-2)
YYL #8-2 (Mut$^+$, mutated EF-2)
X-33 with vector only (Mut$^+$, normal EF-2)

FIG. 10

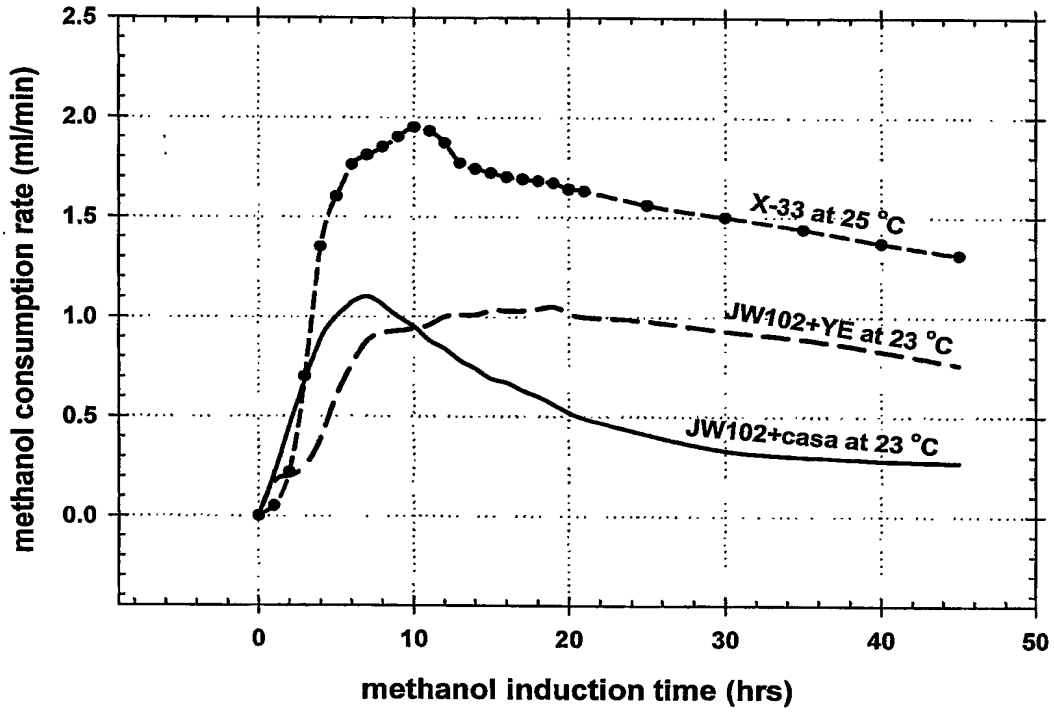

methanol consumption rate (ml/min)
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
methanol induction time (hrs)
X-33 at 25 °C
JW102+YE at 23 °C
JW102+casa at 23 °C

FIG. 11

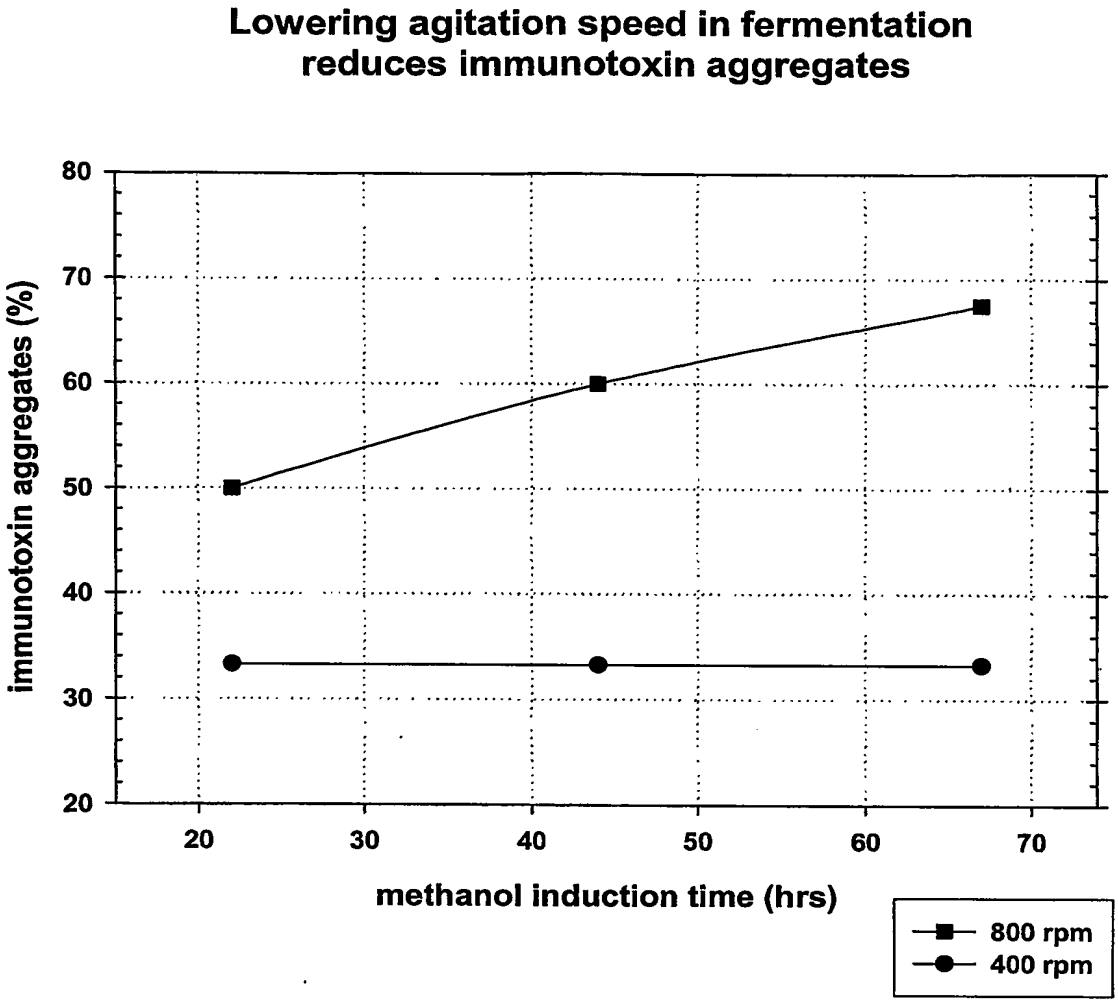

Lowering agitation speed in fermentation reduces immunotoxin aggregates
immunotoxin aggregates (%)
80
70
60
50
40
30
20
20
30
40
50
60
70
methanol induction time (hrs)
800 rpm
400 rpm

Fig. 12

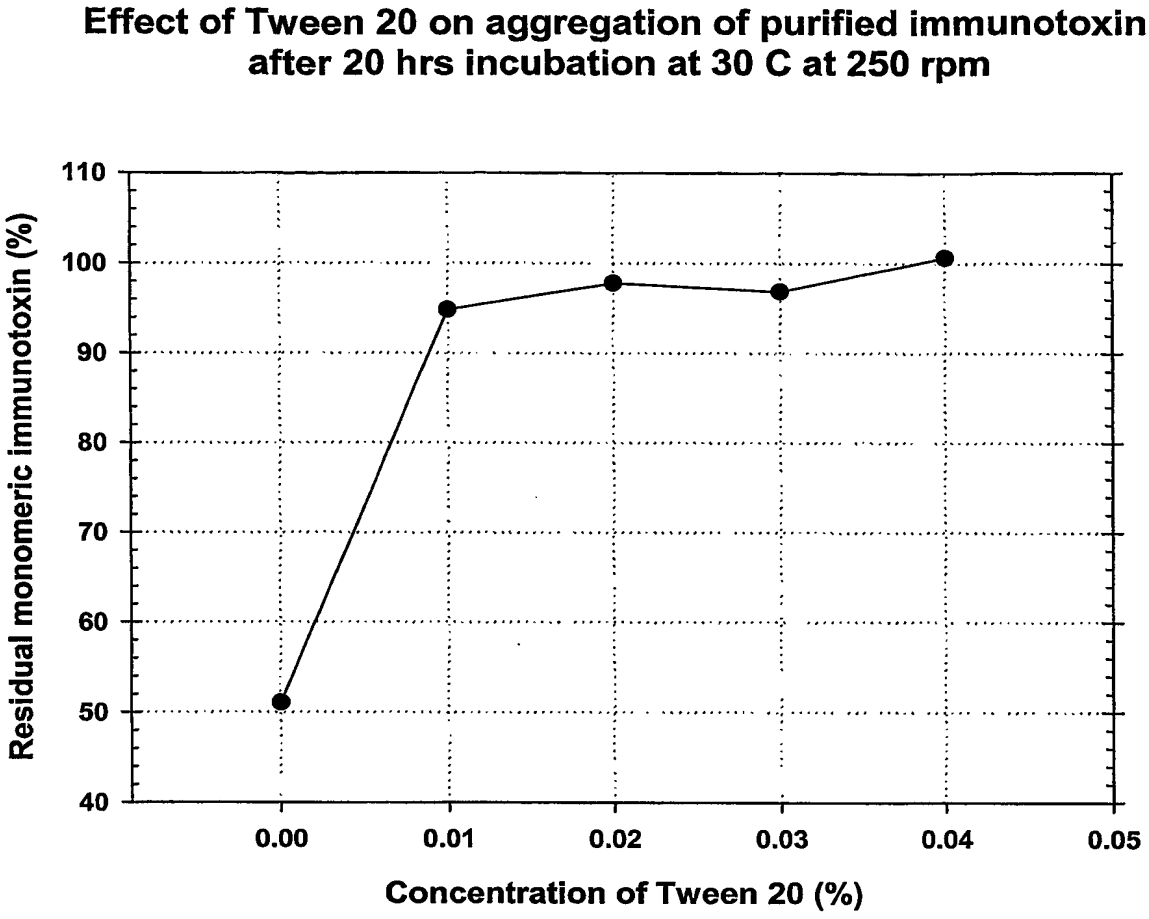

Effect of Tween 20 on aggregation of purified immunotoxin after 20 hrs incubation at 30 C at 250 rpm
Residual monomeric immunotoxin (%)
110
100
90
80
70
60
50
40
0.00
0.01
0.02
0.03
0.04
0.05
Concentration of Tween 20 (%)

Fig. 13

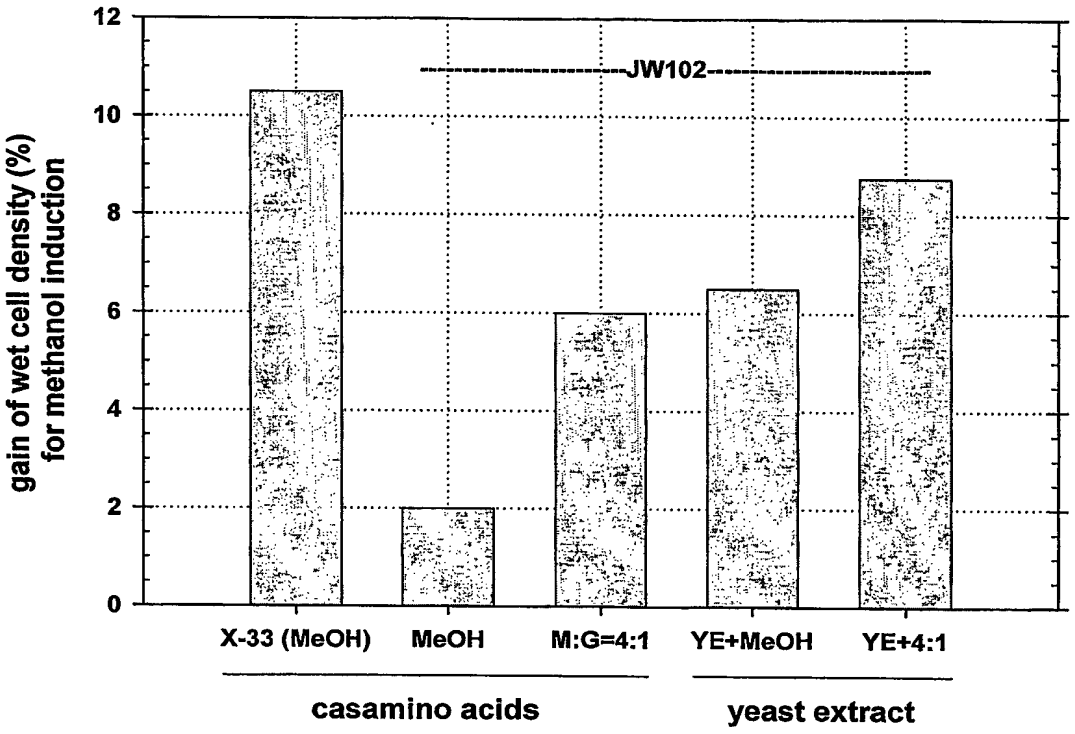

gain of wet cell density (%) for methanol induction
12
10
8
6
4
2
0
JW102
X-33 (MeOH)
MeOH
M:G=4:1
YE+MeOH
YE+4:1
casamino acids
yeast extract

FIG. 14

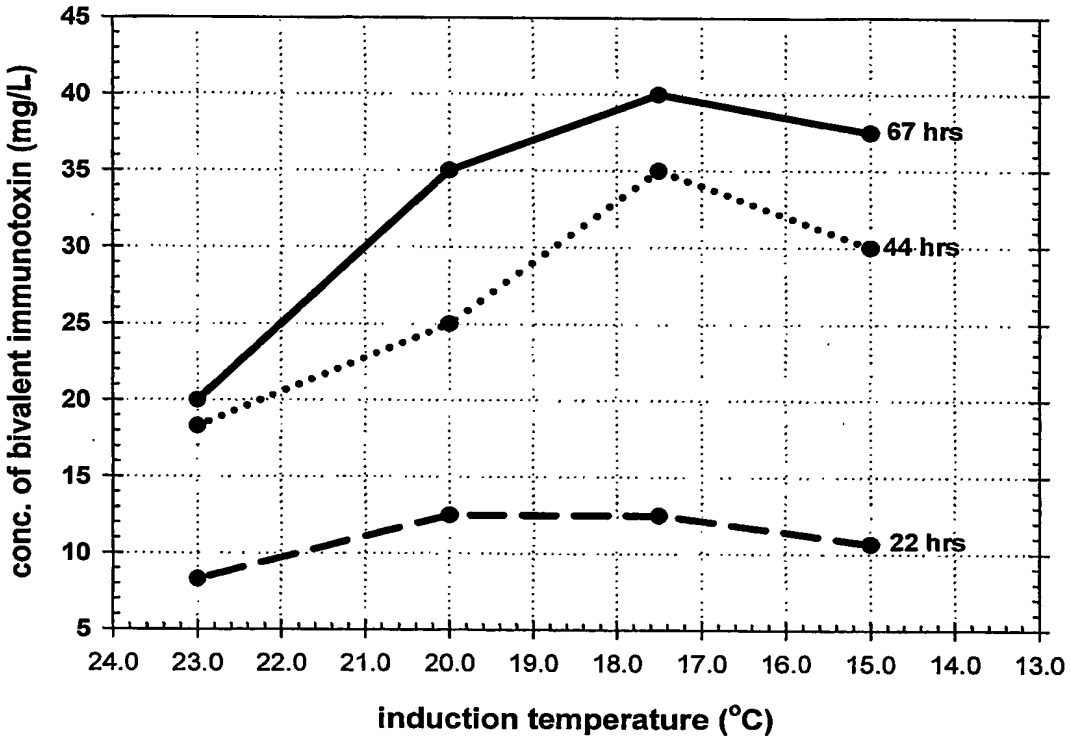

45
40
35
30
25
20
15
10
5
conc. of bivalent immunotoxin (mg/L)
67 hrs
44 hrs
22 hrs
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
induction temperature (°C)

FIG. 15A

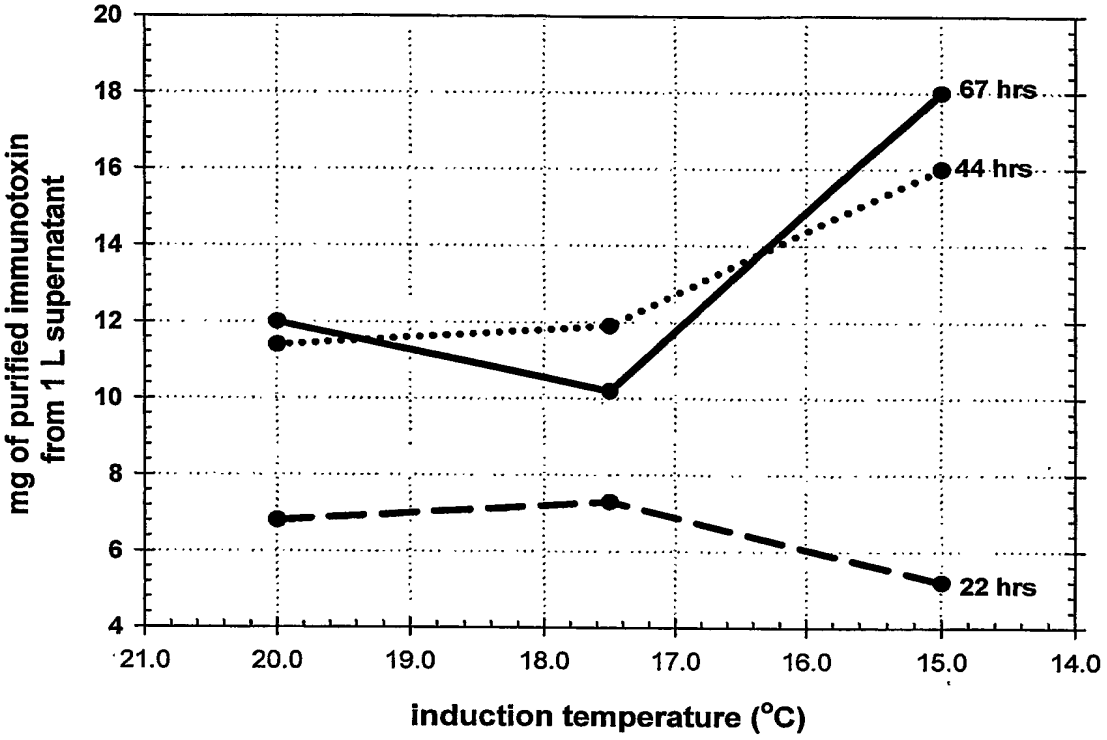

20
18
16
14
12
10
8
6
4
mg of purified immunotoxin from 1 L supernatant
67 hrs
44 hrs
22 hrs
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
induction temperature (°C)

FIG. 15B

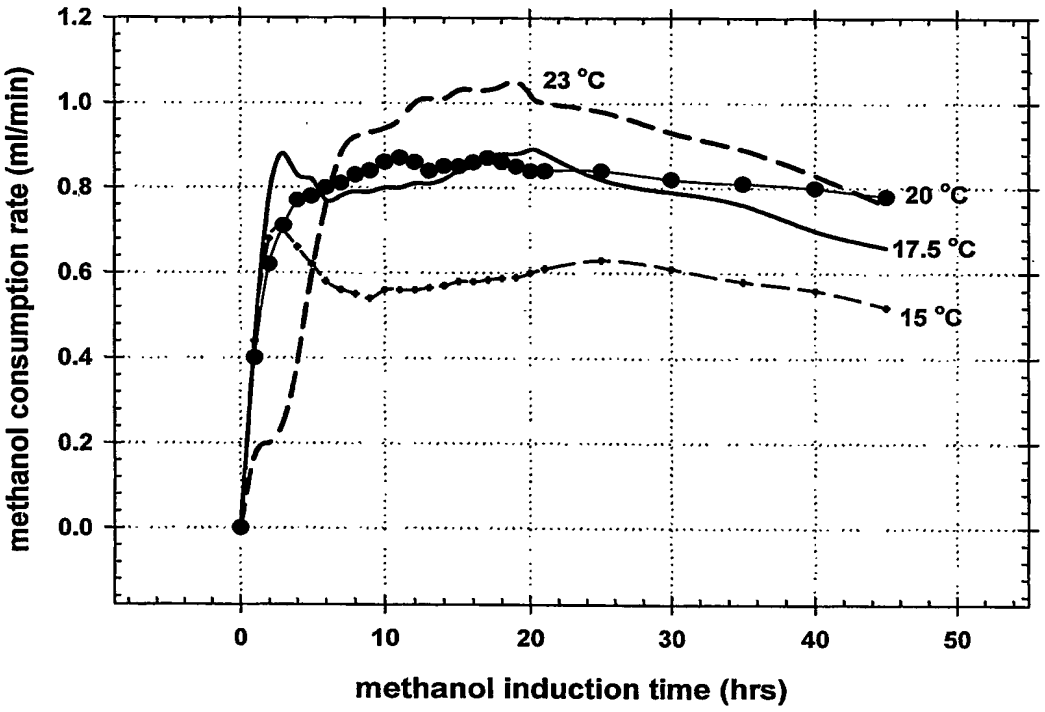

1.2
1.0
0.8
0.6
0.4
0.2
0.0
methanol consumption rate (ml/min)
0
10
20
30
40
50
methanol induction time (hrs)
23 °C
20 °C
17.5 °C
15 °C

FIG. 15C

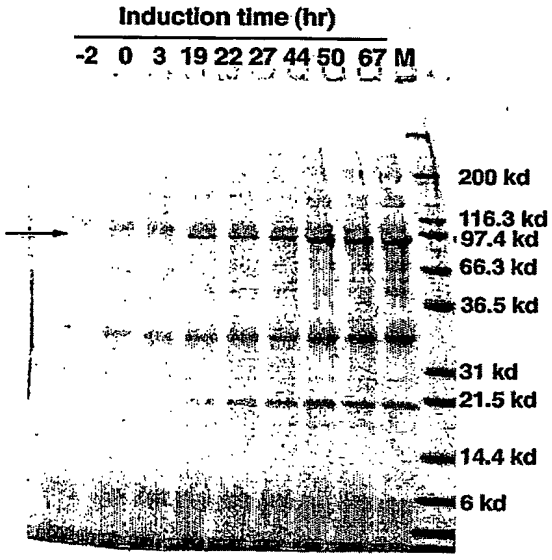

Induction time (hr)
-2 0 3 19 22 27 44 50 67 M
200 kd
116.3 kd
97.4 kd
66.3 kd
36.5 kd
31 kd
21.5 kd
14.4 kd
6 kd

FIG. 16

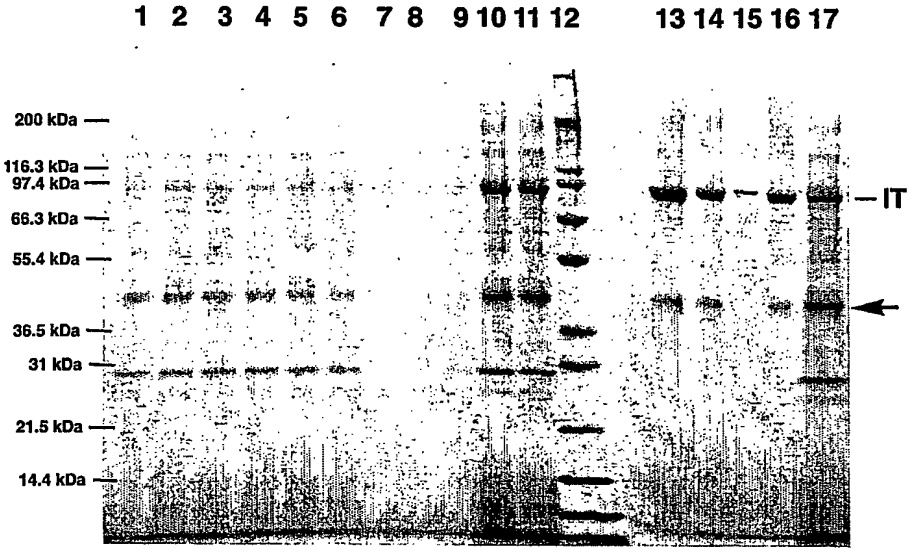

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
200 kDa
116.3 kDa
97.4 kDa
66.3 kDa
55.4 kDa
36.5 kDa
31 kDa
21.5 kDa
14.4 kDa
IT

FIG. 17

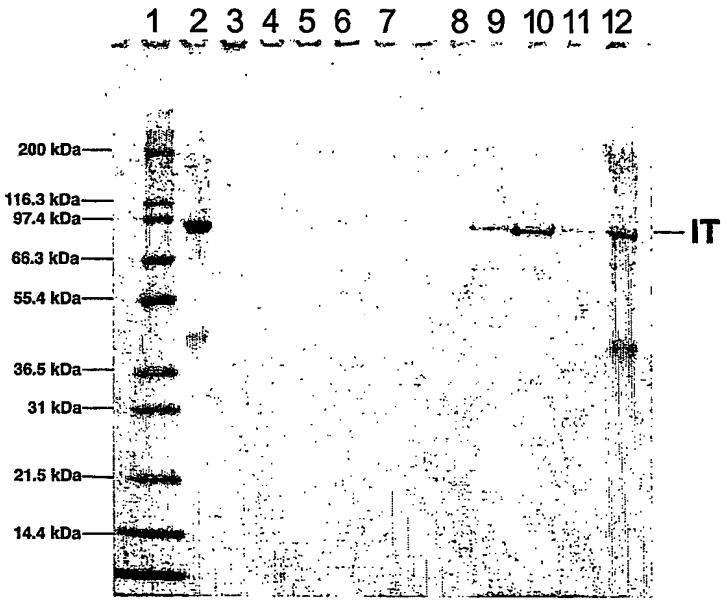
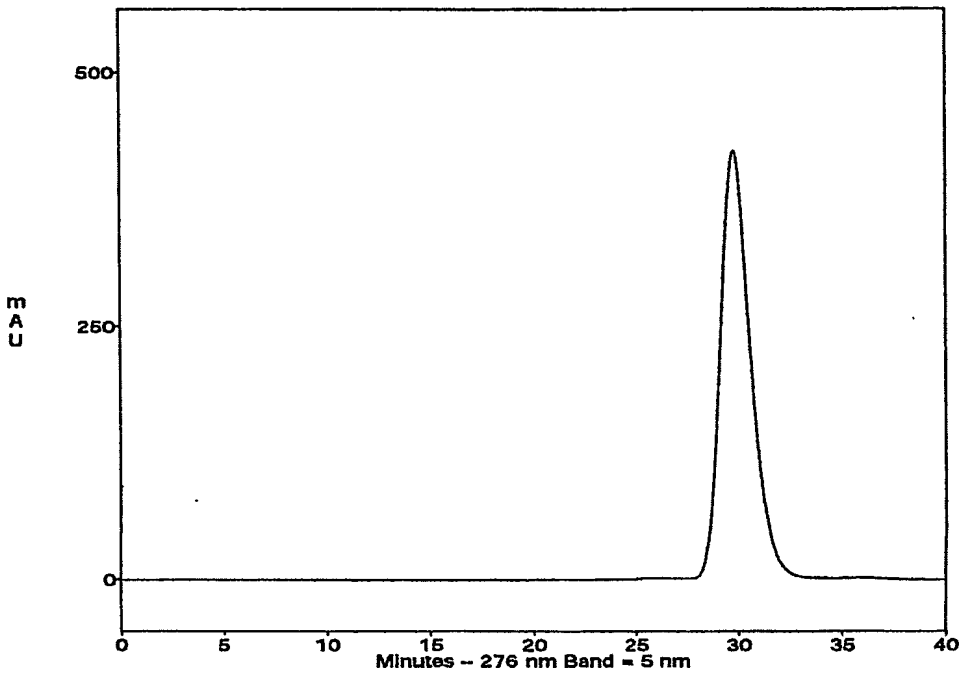

1 2 3 4 5 6 7 8 9 10 11 12
200 kDa
116.3 kDa
97.4 kDa
66.3 kDa
55.4 kDa
36.5 kDa
31 kDa
21.5 kDa
14.4 kDa
IT

FIG. 18

A



B

FIG. 19

Ala Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val Met Glu
1 5 10 15
Asn Phe Ala Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp Ser Ile
20 25 30
Gln Lys Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly Asn Tyr Asp
35 40 45
Asp Asp Trp Lys Gly Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala
50 55 60
Gly Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser Gly Lys Ala Gly Gly
65 70 75 80
Val Val Lys Val Thr Tyr Pro Gly Leu Thr Lys Val Leu Ala Leu Lys
85 90 95
Val Asp Asn Ala Glu Thr Ile Lys Lys Glu Leu Gly Leu Ser Leu Thr
100 105 110
Glu Pro Leu Met Glu Gln Val Gly Thr Glu Glu Phe Ile Lys Arg Phe
115 120 125
Gly Asp Gly Ala Ser Arg Val Val Leu Ser Leu Pro Phe Ala Glu Gly
130 135 140
Ser Ser Ser Val Glu Tyr Ile Asn Asn Trp Glu Gln Ala Lys Ala Leu
145 150 155 160
Ser Val Glu Leu Glu Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln
165 170 175
Asp Ala Met Tyr Glu Tyr Met Ala Gln Ala Cys Ala Gly Asn Arg Val
180 185 190
Arg Arg Ser Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp
195 200 205
Val Ile Arg Asp Lys Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His
210 215 220
Gly Pro Ile Lys Asn Lys Met Ser Glu Ser Pro Ala Lys Thr Val Ser
225 230 235 240
Glu Glu Lys Ala Lys Gln Tyr Leu Glu Glu Phe His Gln Thr Ala Leu
245 250 255
Glu His Pro Glu Leu Ser Glu Leu Lys Thr Val Thr Gly Thr Asn Pro
260 265 270
Val Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln
275 280 285
Val Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Thr Ala Ala
290 295 300
Leu Ser Ile Leu Pro Gly Ile Gly Ser Val Met Gly Ile Ala Asp Gly
305 310 315 320
Ala Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu
325 330 335
Ser Ser Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val
340 345 350
Asp Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn Leu
355 360 365
Phe Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser Pro Gly
370 375 380
His Lys Thr Gln Pro Phe Leu Pro Trp Asp Ile Gln Met Thr Gln Thr
385 390 395 400
Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys
405 410 415

FIG. 20A

Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr Gln Gln Lys
420 425 430
Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His
435 440 445
Ser Gly Val Pro Ser Lys Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
450 455 460
Ser Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe
465 470 475 480
Cys Gln Gln Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly Gly Thr Lys
485 490 495
Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
500 505 510
Gly Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
515 520 525
Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe
530 535 540
Thr Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu
545 550 555 560
Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr Asn
565 570 575
Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
580 585 590
Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val
595 600 605
Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser Asp Trp Tyr Phe
610 615 620
Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
625 630 635 640
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met
645 650 655
Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr
660 665 670
Ile Ser Cys Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr
675 680 685
Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser
690 695 700
Arg Leu His Ser Gly Val Pro Ser Lys Phe Ser Gly Ser Gly Ser Gly
705 710 715 720
Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala
725 730 735
Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly
740 745 750
Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly
755 760 765
Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu
770 775 780
Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly
785 790 795 800
Tyr Ser Phe Thr Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly
805 810 815
Lys Asn Leu Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser
820 825 830

FIG. 20B

Thr Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys
835 840 845
Ser Ser Ser Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp
850 855 860
Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser Asp
865 870 875 880
Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Leu Thr Val Phe Ser
885 890 896

FIG. 20C

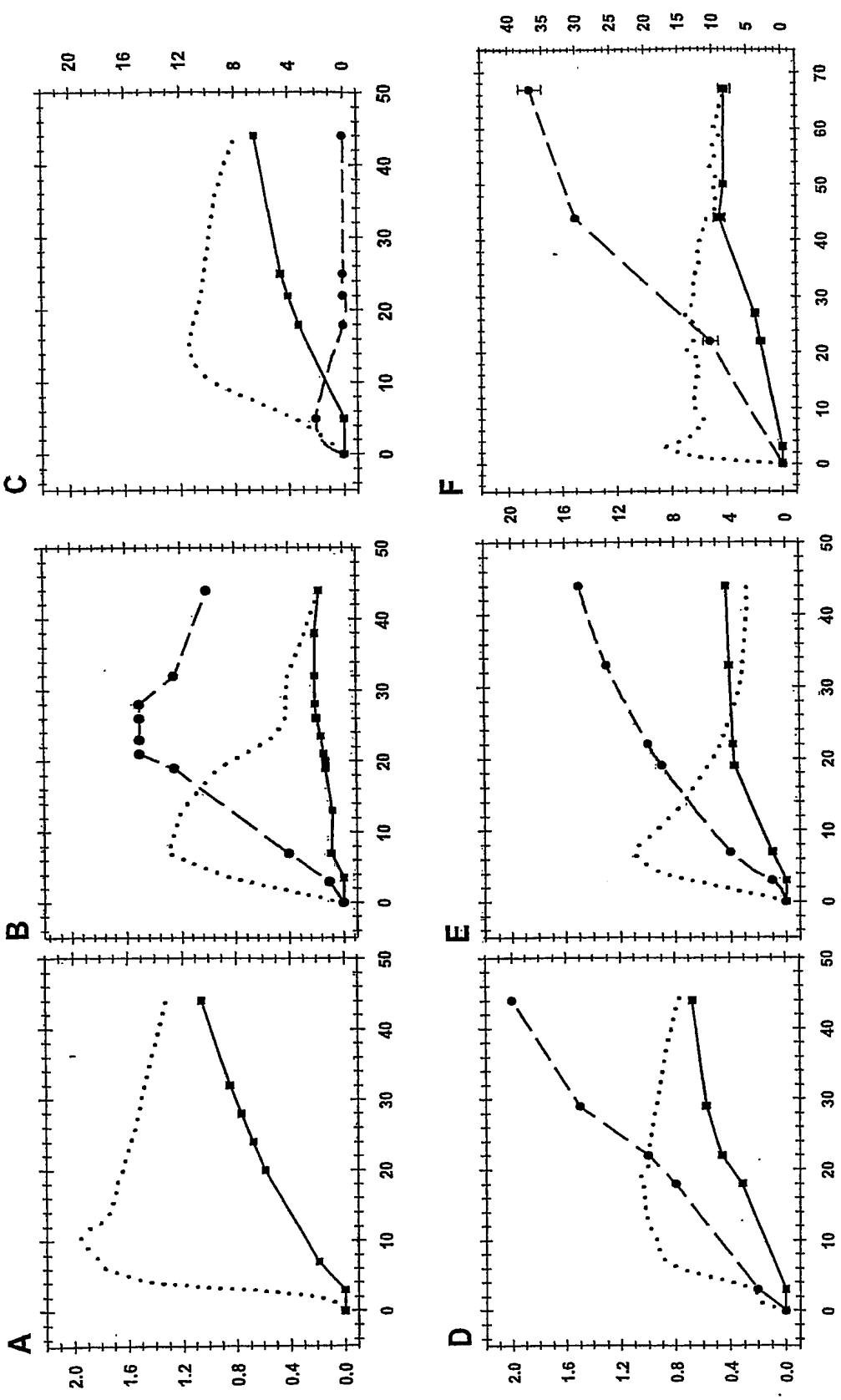
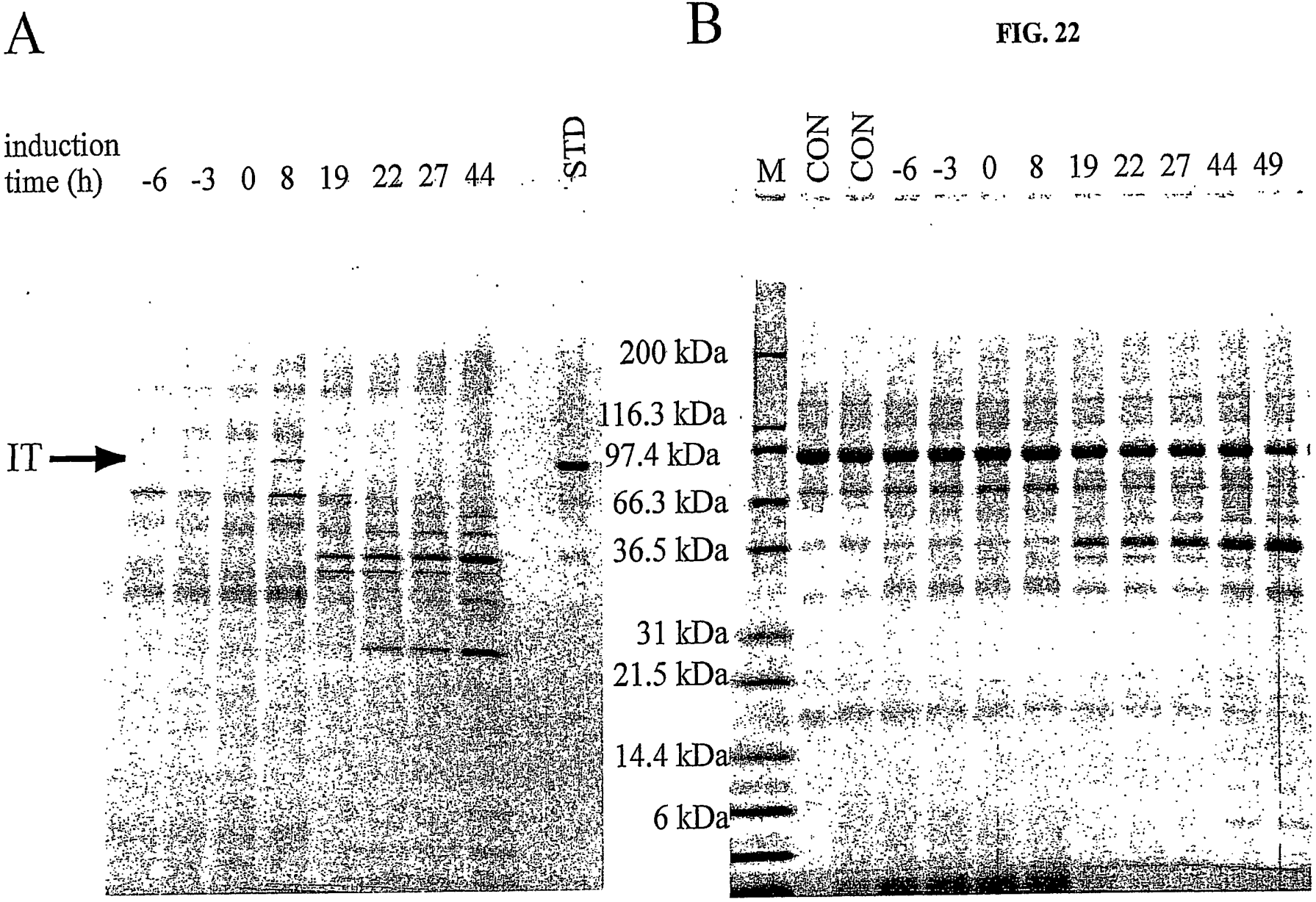

A
B
C
D
E
F
2.0
1.6
1.2
0.8
0.4
0.0
0
10
20
30
40
50
0
4
8
12
16
20
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
60
70
methanol induction time (h)
increase in wet cell density (%) or secretion level
of immunotoxin (mg/L) during methanol induction

FIG. 21

FIG. 22



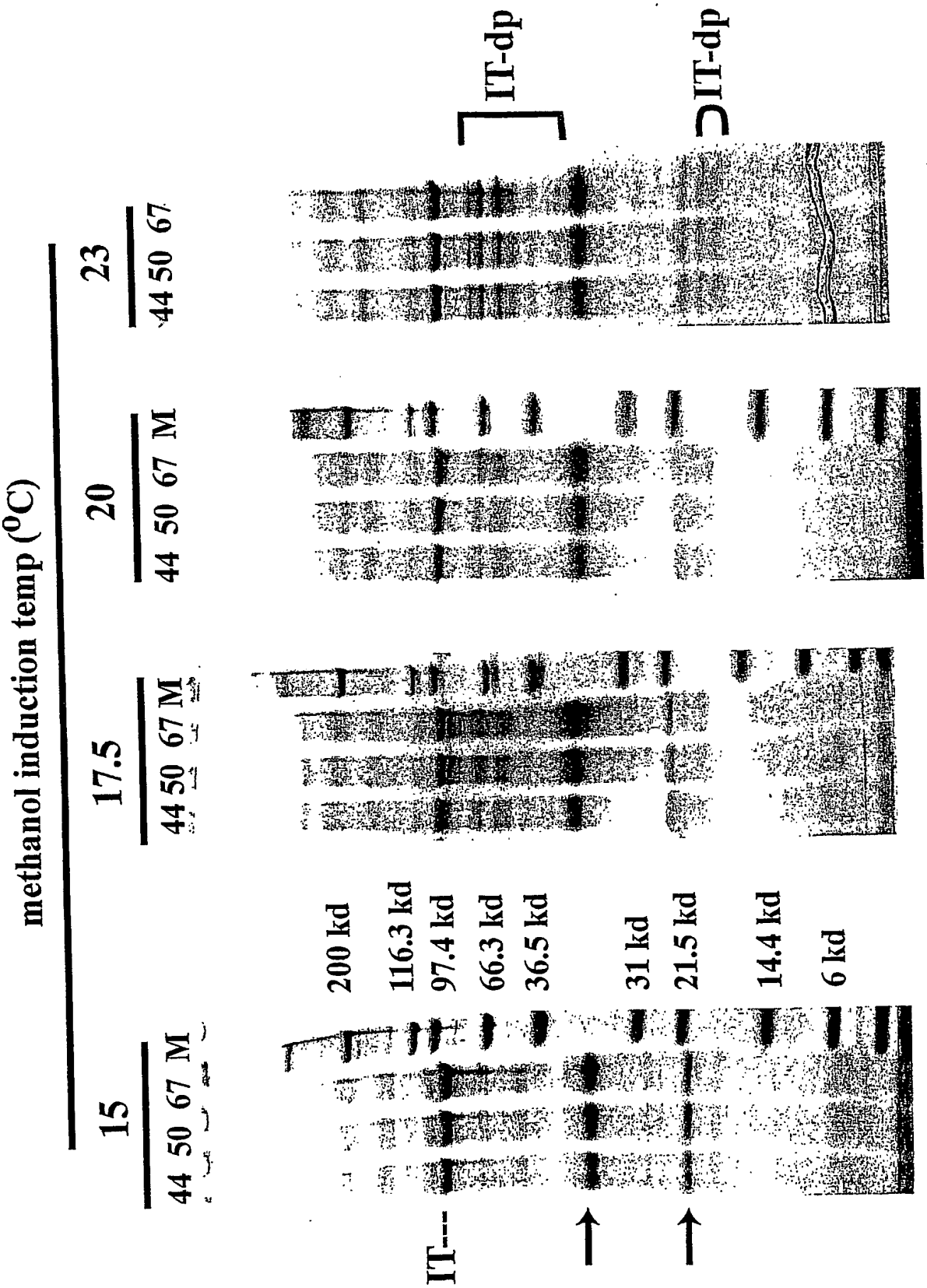
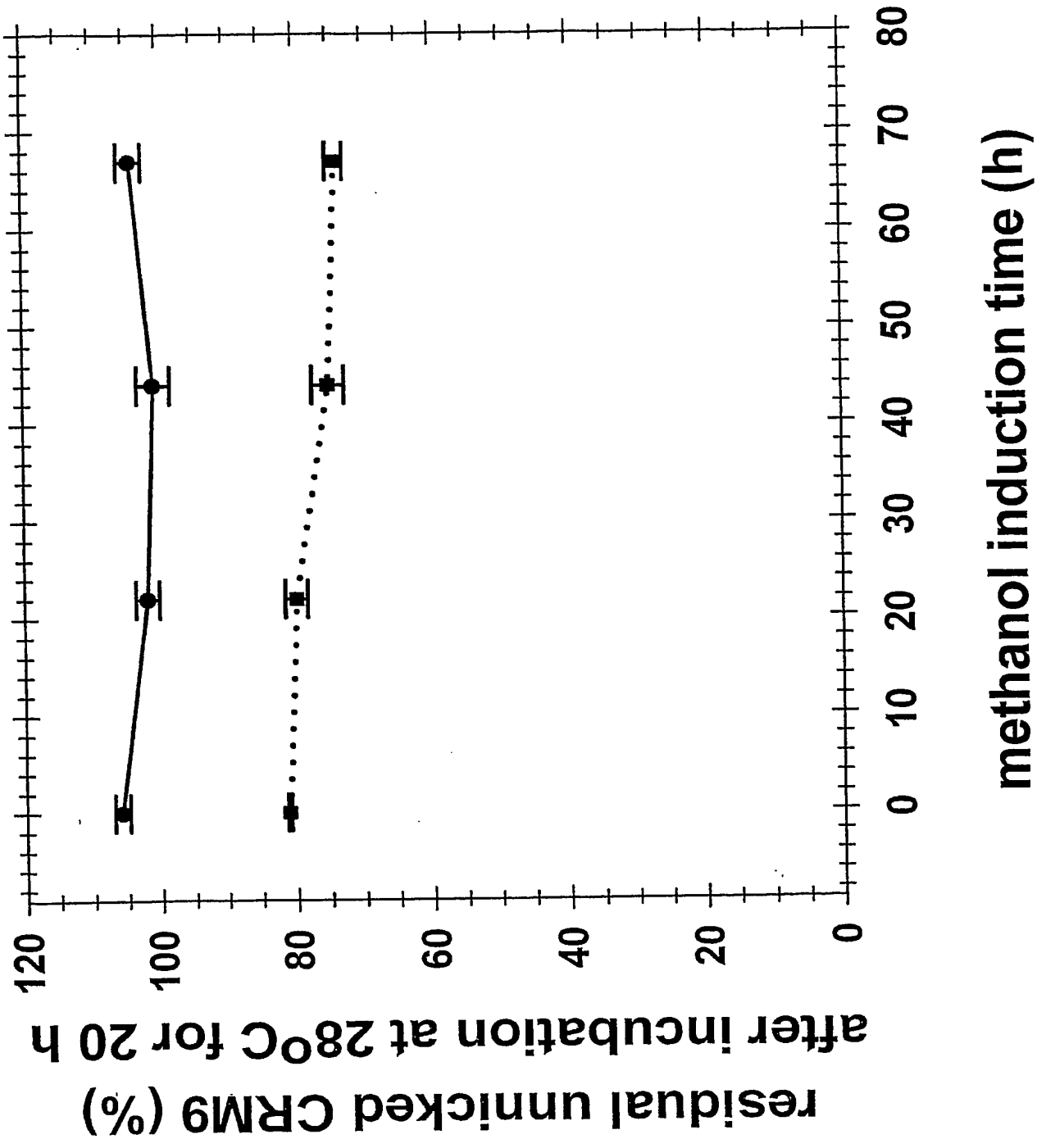


FIG. 24


residual unnicked CRM9 (%)
after incubation at 28°C for 20 h
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
methanol induction time (h)